Ideas crystallized on immunoglobulin superfamily–integrin interactions

Interactions between immunoglobulin superfamily (IgSF) members and integrins are central to lymphocyte homing, leukocyte emigration into tissues at inflammatory sites, and in cell–cell interactions that lead to immune responses. Recent X-ray crystal structures reveal that the interaction of a divalent cation found in the integrin structure with an acidic residue from the IgSF partner may be important in binding.

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Communication between cells is particularly important in the immune system, in which cells are required to locate and migrate to sites of inflammation, and are activated by direct interactions with other cells. The cell–cell interactions important for these processes are mediated by cell-surface molecules that are collectively termed adhesion molecules, which can be broadly grouped into distinct families based on structural similarities. A large number of these molecules fall into the integrin and immunoglobulin superfamilies [1,2]. Both integrins and immunoglobulin superfamily (IgSF) members can bind to a variety of structurally diverse targets, but in many important cell–cell interactions IgSF members interact with members of the integrin family (Fig. 1). Because of the strong similarities within families, it is likely that the interaction surfaces for different integrin–IgSF member pairs have common features. Detailed mutagenesis studies and some new structural information, including the first structure of an integrin domain, have recently revealed some of the features that may be generally important for interactions between molecules of these two families.

The overall architecture of IgSF molecules has been known for some time; they are composed of several copies of a fold that has become classic, the Ig domain. Each Ig domain is ~100–110 amino acids long, and forms a sandwich of two β sheets, consisting of antiparallel β strands of 5–10 amino acids. There is a conserved disulfide bond between the two β sheets in the great majority of domains. All IgSF members that bind integrins contain an integrin-binding site in their first or amino-terminal Ig domain (D1, Fig. 1). VCAM-1 contains an additional integrin-binding site in its fourth Ig domain, which is highly homologous to its first domain; both sites bind to VLA-4. The ICAM-1 molecule binds two different integrins, LFA-1 through D1 and Mac-1 through D3.

The integrin family glycoproteins consist of noncovalently bound αβ heterodimers; they require bound divalent cations to function. Two of the integrins that bind to IgSF members, Mac-1 and LFA-1, have a 180–200 amino-acid insertion in their α subunit, known as the I domain, which for these integrins is thought to be important for their interaction with their IgSF partner. It is interesting, though puzzling, that the structural motifs on ICAM-1, ICAM-3 and VCAM-1 identified by mutagenesis as being important for binding to integrins are all very similar, despite the fact that their integrin targets are structurally different; the amino-terminal domains of both ICAM-1 and ICAM-3 bind to an I-domain-containing integrin, LFA-1, whereas VCAM-1 binds to two integrins that lack an I domain, VLA-4 and $\alpha^4\beta_7$. Thus, VCAM-1 must target a different integrin domain from ICAM-1 and ICAM-3, yet the structural motif important for binding appears to be conserved in the three IgSF members.

The crystallization of domains 1 and 2 of VCAM-1 [3,4], along with extensive mutagenesis of VCAM-1 and ICAMs [5–10] has provided a detailed understanding of the integrin-binding sites found in these molecules. Mutagenesis of domain 1 of VCAM-1 has identified numerous residues important for VCAM-1 function, with mutations centered around Asp40 completely abolishing function. These amino acid substitutions map to or near the protruding C–D loop identified in the crystal structure (Fig. 2). The three-dimensional structure of the C–D loop is important in integrin binding, as a cyclic peptide (Cys-Gln-Ile-Asp-Ser-Pro-Cys) which closely mimics the loop was found to inhibit adhesion of VLA-4-bearing cells to purified VCAM-1, while a linear peptide of identical sequence was inactive [4]. Other residues located outside the C–D loop, especially those on the E–F loop of domain 1, are also thought to be involved in ligand binding. These residues may interact directly with ligand, or may stabilize the C–D loop through the extensive network of hydrogen bonding between the C–D and E–F loops.

Detailed mutagenesis of the fourth Ig domain of VCAM-1 [5,6], and the amino-terminal Ig domains of ICAM-1 and ICAM-3 [8–10] have shown that the critical residues involved in integrin binding are also located in the C–D loop. For the binding of VCAM-1 to VLA-4, the key residue in both domains 1 and 4 of VCAM-1 is an aspartate, whereas for the amino-terminal domains of ICAM-1

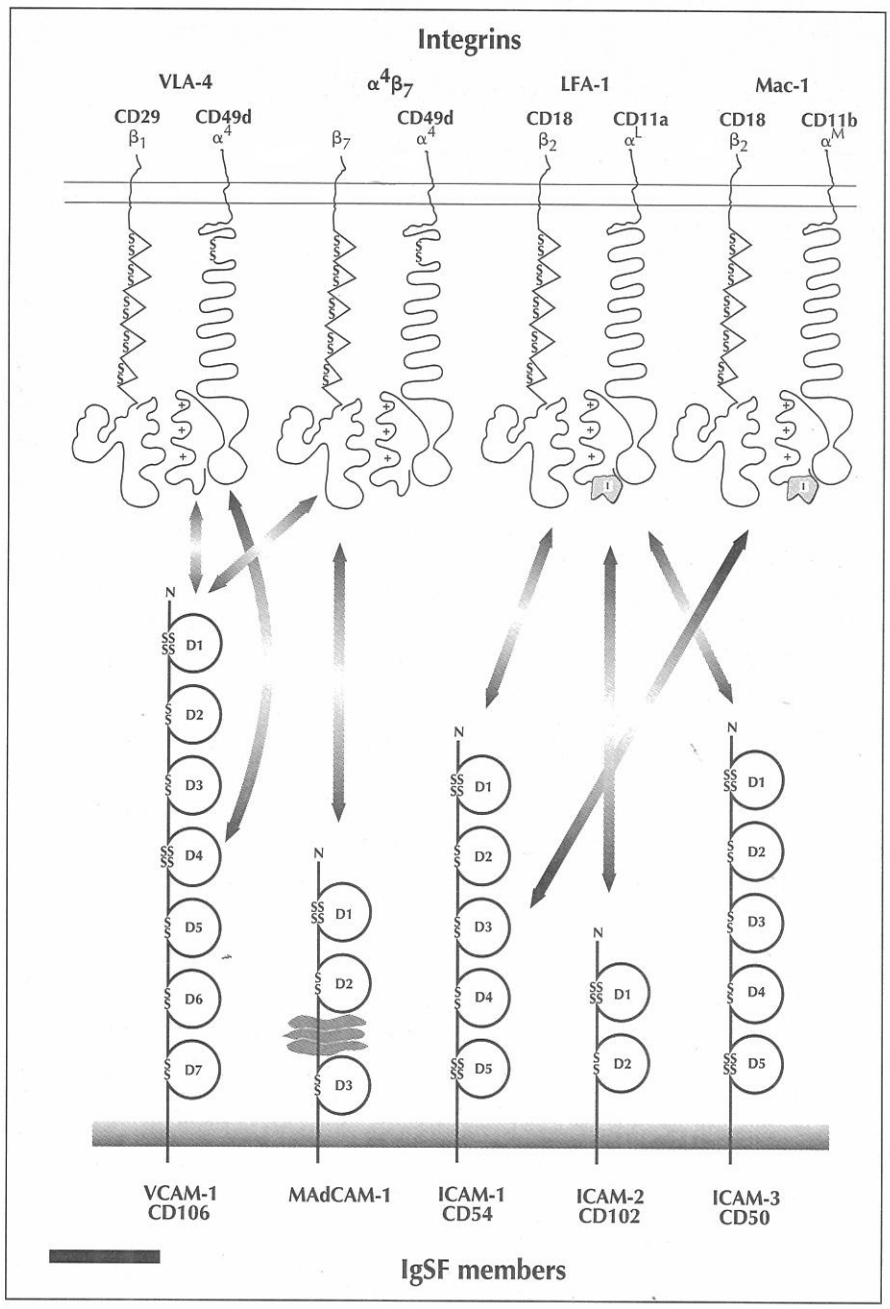

Fig. 1. Schematic diagram of integrin and IgSF members that form adhesion ligand pairs. These interactions are important in several biological processes. For example, the adhesion of neutrophils to vascular endothelium, an early step in the process of their migration through the wall of the blood vessel to the site of an infection, is mediated by LFA-1 and Mac-1 binding to ICAM-1, while VLA-4 interactions with VCAM-1 are important in the homing of mature, activated T cells to tissues. For reviews on the biological functions of these interactions, see [1,2]. Arrows indicate the integrin–IgSF interactions that have been demonstrated. The overall size and shape of the integrins and IgSF members are shown to scale, based on electron microscopy (bar 10 nm). It is as yet unclear how the I domain may fold up with the rest of the globular head. Divalent cation binding sites are symbolized by '+'. Disulfide-bonded cysteines are denoted in the IgSF members by 'SS'. In the integrin α-subunits SS denotes a disulphide bond that bridges a cleavage site; in the β-subunits it designates a cysteine-rich region. The serine/threonine-rich mucin domain of MAdCAM-1, an IgSF member which guides lymphocyte entry into mucosal lymphoid tissue such as the gut is also indicated.

and ICAM-3 this residue is a glutamic acid. Sequence analysis of the C–D loops in Ig domains involved in integrin binding reveals strong similarities, which are well conserved across species. From these data, a tentative consensus sequence on the IgSF molecule of Ile/Leu-Asp/Glu-Ser/Thr-Pro(Xaa)-Leu(Xaa) for integrin–IgSF interactions has been proposed (Xaa indicates that alternative residues are used in some cases) [5].

Although the integrin-binding sites on the IgSF members have been extensively investigated, the corresponding regions on the integrins have only recently been defined. The α subunits of integrins that interact with IgSF members (α^4, α^L and α^M) contain three putative cation-binding repeats in the globular head region (Fig. 1); α^L and α^M also contain an I domain at their amino terminus. The I domain (sometimes also referred to as an A domain) is found in one or more copies in a wide range of proteins involved in cell–cell, cell–matrix, and matrix–matrix interactions.

The recent determination of the crystal structure of the Mac-1 I domain [11] has offered insight into how the C–D loop, and more specifically the key acidic aspartate or glutamate residue, is involved in integrin binding. The I domain is an open sheet structure with alternating amphipathic α helices and central hydrophobic β strands, and contains a Mg^{2+} coordination site on its surface in a motif termed a metal ion-dependent adhesion site (MIDAS; see Fig. 3). The structure that the I domain adopts is present in a wide variety of intracellular enzymes, and the crevice at the top of the β sheet forms a ligand-binding site, which often includes a metal ion, in all known proteins of this class. One of the six Mg^{2+} coordination positions is free to bind ligand; in the crystal it adventitiously ligates a glutamic acid residue from a

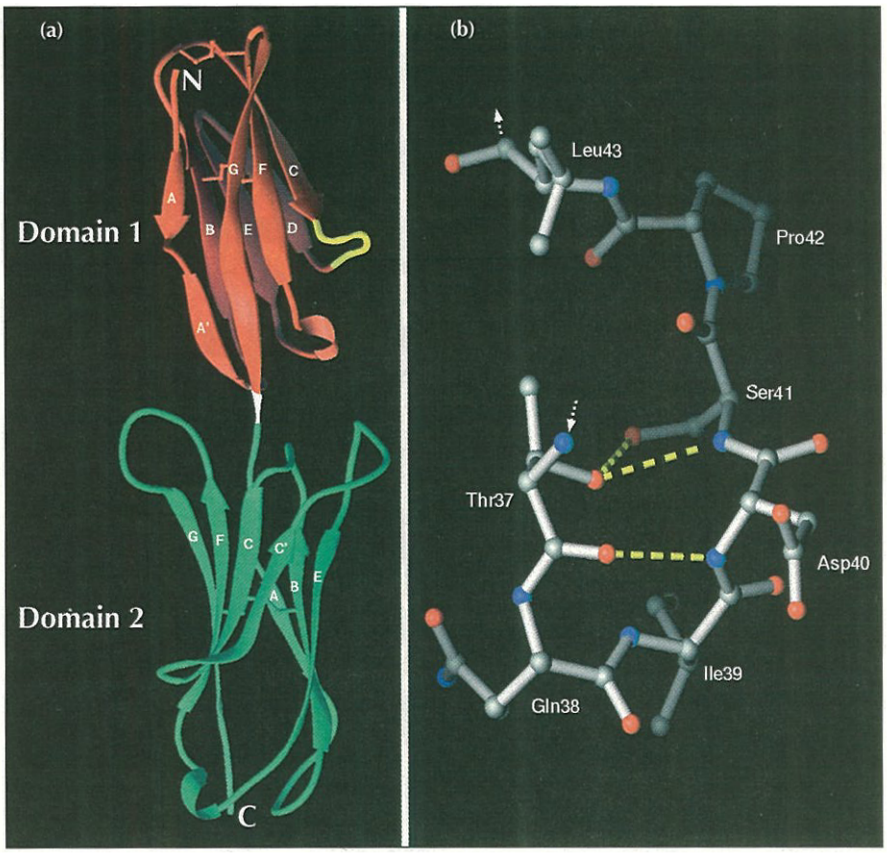

Fig. 2. Structure of VCAM-1 domains 1–2 based on crystallographic data. **(a)** Schematic representation of the molecule, with the amino-terminal part of the C–D loop (residues 37–43) highlighted in yellow. **(b)** Atomic structure of residues 37–43. The structure is stabilized by three internal hydrogen bonds (shown in yellow), including one that is found in a β-turn. Arrows indicate the direction of the polypeptide. Figure reproduced by kind permission of Osborn and colleagues [4].

neighbouring I domain in the lattice. The importance of the Mg^{2+} coordination site within the I domain is demonstrated by the fact that disruption of the residues involved in metal coordination in the I domain affect not only Mg^{2+} ion binding, but also ligand binding for both β_2 and β_1 integrins (when their α subunits contain I domains) [11–15]. A direct role for this Mg^{2+}-binding site in integrin binding is strongly suggested by the extra, 'available' coordination site revealed in the crystal structure. It is tempting to speculate that the sixth coordinating residue is contributed by an acidic aspartate or glutamate residue from the interacting IgSF domain. Recent mutagenesis studies on LFA-1 also support a central role for the Mg^{2+} ion in the I domain in binding to ICAM-1 [16]. Residues responsible for species-specific binding of human, but not mouse, LFA-1 to human ICAM-1 were mapped, and found to surround the Mg^{2+}-binding site on the I domain, defining a ligand binding interface on an integrin.

The conserved features in the MIDAS motif are the sequence Asp-Xaa-Ser-Xaa-Ser within the I domain (residues 140–144) and non-contiguous threonine (Thr209) and aspartate (Asp242) residues. These form direct or indirect coordinations with the Mg^{2+} ion, and, together with water molecules that they bind, fulfill five of the six octahedral coordination positions (Fig. 3b). Analysis of known I domains suggest that the majority possess MIDAS motifs. Not all integrin-Ig interactions involve an I domain, as demonstrated by $\alpha^4\beta_1$ interaction with VCAM-1. However, all β subunits do possess an Asp-Xaa-Ser-Xaa-Ser motif, and mutagenesis of this region in β_1 and β_3 integrins abolishes their interaction with extracellular matrix proteins [17,18]. This motif in the β subunit has been proposed to be present in a region that folds similarly to the I domain [11]. When all of this information is combined, an attractive model of the interaction between integrins and IgSF members at the molecular level emerges. The integrin molecule coordinates five of six coordination sites on a metal ion through a MIDAS motif in either its I domain or its β subunit, while an acidic residue (Asp or Glu), prominently protruding on a C–D loop from the IgSF molecule, acts to coordinate the sixth and final metal ion site.

There is a strong precedent for conserved amino acid motifs that are important for integrin binding to other targets. Many integrins bind to extracellular matrix proteins through either Arg–Gly–Asp or Leu–Asp–Val motifs found on the matrix proteins, with binding being dependent in both cases on the aspartate residue [2]. Thus, similar motifs are important in integrin–matrix and integrin–IgSF interactions; both require a MIDAS motif on the integrin, a metal ion, and an acidic residue on the integrin ligand. All of these interactions, therefore, may involve a divalent cation bound to an integrin that ligates an acidic residue from the binding partner.

The presence of the integrin-binding motif is not the sole determinant of integrin-binding specificity, however, as residues outside this motif are required for binding, and exchange of this motif in domain 1 of VCAM-1 with that in ICAM-1 did not alter VCAM-1 specificity for VLA-4 [6]. Similarly, the region of the

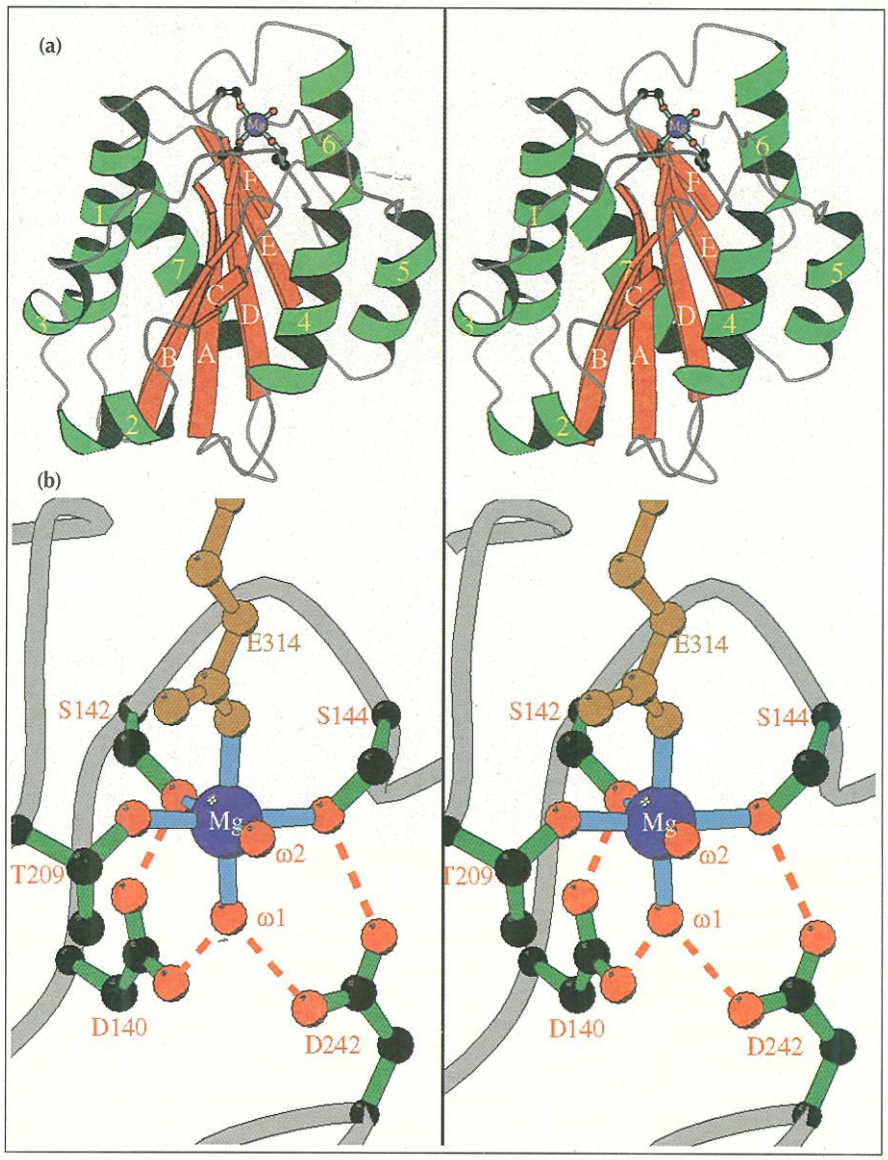

Fig. 3. Structure of the Mac-1 I domain. **(a)** Schematic stereo representation of the I domain, with the coordinated Mg^{2+} ion shown in blue. **(b)** Close-up of the MIDAS site. Coordinating oxygen atoms are shown in red, and hydrogen bonds are shown by dashed red lines. Glu314 from another I domain molecule is shown in gold (E314). The protein backbone is shown schematically as a gray ribbon. Figure reproduced by kind permission of Lee *et al.* [11].

integrin that is involved in adhesion may vary slightly depending on its ligand. For example, various monoclonal antibodies that bind to different regions of the LFA-1 I-domain differ in their ability to affect LFA-1 binding to ICAM-1, -2, or -3 [19,20]. Lastly, not all IgSF–integrin interactions are dependent on the integrin-binding motif described above, as the Mac-1 binding site on ICAM-1, the third Ig domain [9], contains no integrin-binding consensus sequence. While mutagenesis of ICAM-1 has revealed that several acidic residues in the third Ig domain, including an aspartate residue in the C–D loop, are crucial for Mac-1 binding, the glycosylation state of ICAM-1 is also important, as mutations that destroy sites for N-linked glycosylation and agents that interfere with carbohydrate processing result in increased Mac-1 binding [9].

Adhesive interactions vary in requirement for divalent cations. Those involving heterophilic binding of one IgSF member to another IgSF member, or homophilic IgSF interactions, do not require divalent cations for binding activity, whereas both the selectin and cadherin families of adhesion molecules require calcium ions. The recent crystallization of a cadherin suggests that the Ca^{2+} ions are important not in the ligand interface, but in the interface between different domains of the same protein, providing rigidity to an adhesion 'zipper' [21]. The separate crystallization of regions important in ligand binding in an IgSF molecule and an integrin has offered insight into how members of these two families might mediate adhesive interactions, and has suggested that cations are important in this process. However, on exact understanding of how IgSF members interact with integrins awaits the crystal structure of appropriate integrin: IgSF or I domain: IgSF co-complexes.

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